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MUTAGENIC POTENTIAL OF METHYL-N,N' DIHEXYLETHYLENE
DIAMINE MONOCARBAMATE (U) LETTERMAN ARMY INST OF
RESEARCH PRESIDIO OF SAN FRANCISCO CA

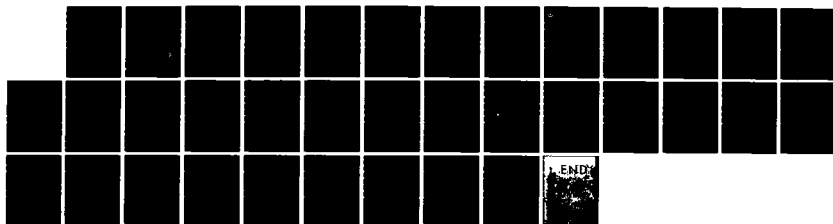
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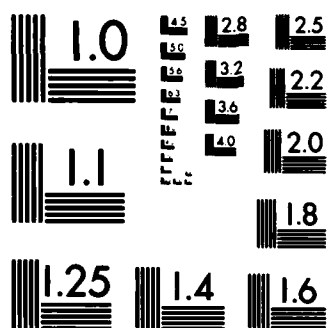
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INSTITUTE REPORT NO. 145

MUTAGENIC POTENTIAL OF:

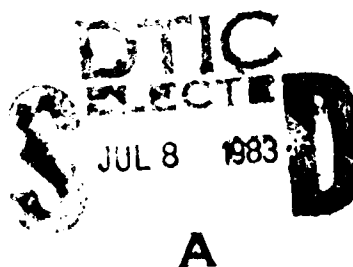
methyl N, N' dihexylethylene diamine monocarbamate (CHR 4)
1,2,3,4 tetrahydro-6-methyl-1-(3-methyl-1-oxo-2-butenyl)
quinoline (CHR 6)

LEONARD J. SAUERS, MS, SP5

and

JOHN T. FRUIN, DVM, PhD, COL VC

TOXICOLOGY GROUP,
DIVISION OF RESEARCH SUPPORT



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MAY 1983

Toxicology Series 48

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19. KEY WORDS (Continue on reverse side if necessary and identify by block number) Mutagenicity, Toxicology, Ames Assay, Methyl N, N' Dihexylethylene Diamine Monocarbamate (CHR4), 1,2,3,4-Tetrahydro-6-Methyl-1- (3-Methyl-1-Oxo-2-Butenyl) Quinoline (CHR6)		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The mutagenic potential of methyl N, N' dihexylethylene diamine monocarbamate (CHR4*) and 1,2,3,4-tetrahydro-6-methyl-1-(3-methyl-1-oxo-2-butenyl) quinoline (CHR6*) was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Assay. Tester strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 were exposed to doses ranging from 0.1% to 3.2 X 10 ⁻⁵ % of an undiluted sample of CHR4 and 1% to 3.2 X 10 ⁻⁴ % of an undiluted sample of CHR6. Results of the Ames Assay indicate that these substances do not have mutagenic potential. *Code number for compound		

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ABSTRACT

The mutagenic potential of methyl N, N' dihexylethylene diamine monocarbamate (CHR4*) and 1,2,3,4-tetrahydro-6-methyl-1-(3-methyl-1-oxo-2-butenyl) quinoline (CHR6*) was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Assay. Tester strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 were exposed to doses ranging from 0.1% to $3.2 \times 10^{-5}\%$ of an undiluted sample of CHR4 and 1% to $3.2 \times 10^{-4}\%$ of an undiluted sample of CHR6. Results of the Ames Assay indicate that these test substances do not have mutagenic potential.

*Code number for compound

KEY WORDS: Mutagenicity, Toxicology, Ames Assay, Methyl N, N' Dihexylethylene Diamine Monocarbamate (CHR4), 1,2,3,4-Tetrahydro-6-Methyl-1- Oxo-2-Butenyl) Quinoline (CHR6).



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PREFACE

TYPE REPORT: Ames Assay GLP Study Report

TESTING FACILITY: US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129

SPONSOR: Same as above

PROJECT: 3M162770A871 Development of Repellents Against Medically
Important Arthropods, WU 201, APC TL01

GLP STUDY NUMBER: 82024

STUDY DIRECTOR: COL John T. Fruin, D.V.M., PhD, VC, Diplomate of
American College of Veterinary Preventive Medicine

PRINCIPAL INVESTIGATOR: SP5 Leonard J. Sauers, MS

REPORT AND DATA MANAGEMENT: A copy of the final report, retired SOPs,
raw data, and chemical, analytical, stability,
and purity data for the test compound will be
retained in the LAIR Archives.

TEST SUBSTANCE: Methyl-N, N' Dihexylethylene Diamine Monocarbamate (CHR4)
and
1,2,3,4-Tetrahydro-6-Methyl-1-(3-Methyl-1-Oxo-2-Butenyl
Quinoline (CHR6)

INCLUSIVE STUDY DATES: 10 August - 10 September 1982

OBJECTIVE: To determine the mutagenic potential of the compounds CHR4
and CHR6 using the Ames Assay. Tester strains TA 98, TA 100,
TA 1535, TA 1537 and TA 1538 were used. The test substance
was dissolved in ethanol and this diluent was checked for
sterility.

ACKNOWLEDGMENTS

The authors wish to thank John Dacey, Carolyn Lewis, MS, and SP4 Thomas Kellner, BS for their assistance in performing the research.

Signatures of Principal Scientists involved
in the Study

We, the undersigned, believe the study number 82024 described
in this report to be scientifically sound and the results and
interpretation to be valid. The study was conducted to comply, to
the best of our ability, with the Good Laboratory Practice
Regulations outlined by the Food and Drug Administration.

<i>Leonard J. Sauer</i> 2 May 83	<i>John T. Fruin</i> 2 May 83
LEONARD J. SAUERS, MS / DATE	JOHN T. FRUIN, DVM, PhD / DATE
SP5, USA	COL, VC
Principal Investigator	Study Director



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PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA

29 Mar 83

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LAIR GLP study 82024 the following inspections were made:

9 Aug 82
18 Aug 82
23 Aug 82

The report and raw data for this study were audited on 25 Mar 83.

Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the Oct 82 report to management and the Study Director.

Nelson R. Powers
NELSON R. POWERS, Ph.D.
CPT, MSC
Quality Assurance Officer

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MUTAGENIC POTENTIAL OF: methyl N, N' dihexylethylene diamine monocarbamate (CHR 4)
and 1,2,3,4 tetrahydro-6-methyl-1-(3-methyl-1-oxo-2-butenyl) quinoline (CHR 6)--Sauers and Fruin

Rationale for using the Ames Assay

The Ames Salmonella/Mammalian Microsome Mutagenicity Test is one of a standard bank of tests used by our laboratory for the assessment of the mutagenic potential of a test substance. It is a short-term screening assay, which we use for the prediction of potential mutagenic agents in mammals. It is inexpensive when compared to in vivo tests, yet is highly predictive and reliable in its ability to detect mutagenic activity and therefore carcinogenic probability (1). It relies on basic genetic principles and allows for the incorporation of a mammalian microsomal enzyme system to increase sensitivity through enzymatically altering the test substance into an active metabolite. It has proven highly effective in assessing human risk (1).

Description of Test (Rationale for the selection of strains)

The test was developed by Bruce Ames, Ph.D. from the University of California-Berkeley. The test involves the use of several different genetically altered strains of Salmonella typhimurium, each with a specific mutation in the histidine operon (2). The test substance demonstrates mutagenic potential if it is able to revert the mutation in the bacterial histidine operon to the wild type and reestablish prototrophic growth within the test strain. This reversion also can occur spontaneously due to a random mutational event. If, after adding a test substance, the number of revertants is significantly greater than the spontaneous reversion rate, then the test substance physically altered the locus involved in the operon's mutation and is able to induce point mutations (2).

In order to increase the sensitivity of the test system, other mutations in the Salmonella are used (2). To insure a higher probability of uptake of test substance, the genome for the lipopolysaccharide layer (LP) is mutated and, therefore, larger molecules are allowed to enter the bacteria. Each strain has another induced mutation which causes loss of excision repair mechanisms.

A mammalian microsomal enzyme system is incorporated since many chemicals are not by themselves mutagenic but have to be activated by an enzymatic process. These microsomal enzymes are obtained from livers of rats induced with Aroclor 1254; the enzymes allow for the expression of the metabolites which would occur in the mammalian system. This activated rat liver microsomal enzyme homogenate is termed S-9.

Description of Strains (History of the strains used method to monitor the integrity of the organisms, and data pertaining to current and historical control and spontaneous reversion rates)

The test consists of using five different strains of Salmonella typhimurium that are unable to grow in absence of histidine because of a specific mutation in the histidine operon. This histidine requirement is verified by attempting to grow the tester strains on minimal glucose agar (MGA) plates, both with and without histidine. The dependence on this amino acid is shown when growth occurs only in its presence. The plasmids in strains TA 98 and TA 100 contain an ampicillin resistant R factor. Strains deficient in this plasmid demonstrate a zone of inhibition around an ampicillin impregnated disc. The alteration of the LP layer allows uptake by the Salmonella of larger molecules. If a crystal violet impregnated disc is placed onto a plate containing any one of the bacterial strains, a zone of growth inhibition will occur because the LP layer is altered. The absence of excision repair mechanisms can be determined by using ultraviolet (UV) light. These mechanisms function primarily by repairing photodimers between pyrimidine bases; exposure of bacteria to UV light will activate the formation of these dimers and cause cell lethality, since excision of these photodimers can not be made. The genetic mutation resulting in UV sensitivity also induces a dependence by the Salmonella to biotin. Therefore, this vitamin must be added. In order to prove that the bacteria are responsive to the mutation process, positive controls are run with known mutagens. If after exposure to the positive control substance, a revertant count is obtained which is greater than twice the spontaneous reversion rate, then the bacteria are adequately responsive. Sterility controls are performed to determine the presence of contamination. Sterility of the test compound is also confirmed in each first dilution. Verification of the tester strains occurs simultaneously with the running of each assay. The value of the spontaneous reversion rate is obtained by using the same inoculum of bacteria that is used in the assay (3).

Strains were obtained directly from Dr. Ames, University of California-Berkeley, propagated and then maintained at -80°C in our laboratory. Before any substance was tested, quality controls were performed on the bacterial strains to establish the presence of their special features and also to determine the spontaneous reversion rate (2). Records are maintained of all the data to determine if deviations from the set trends have occurred. These records are kept in the archives of the Quality Assurance Unit.

In this series of tests for the detection of mutagenic potential of different agents, we compare the spontaneous reversion values with our own historical values and those cited by Ames et al (2). Our conclusions are based on the spontaneous reversion rate compared to the experimentally induced rate of mutation. When operating effectively, these strains detect substances that cause base pair mutations (TA 1535, TA 100) and frameshift mutations (TA 1537, TA 1538, and TA 98).

Objective of Study

The objective of the study is to determine the mutagenic potential of the compounds CHR4 and CHR6 by using the Ames Assay. Tester strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 were used. The test substances were dissolved in ethanol and this diluent was checked for sterility.

METHODS (3)

Rationale for Dosage Levels and Dose Response Tabulations

To insure readable and reliable results, a sublethal concentration of the test substance had to be determined. This toxicity level was found by using MGA plates, various concentrations of the substance, and approximately 10^8 cells of TA 100 per plate, unless otherwise specified. Top agar containing trace amounts of histidine and biotin were placed on MGA plates. TA 100 is used because it is the most sensitive strain. Strain verification was confirmed on the bacteria, along with a determination of the spontaneous reversion rate. After incubation, the growth was observed on the plates. (The auxotrophic Salmonella will replicate a few times and potentially express a mutation. When the histidine and biotin supplies are exhausted, only those bacteria that reverted to the prototrophic phenotype will continue to reproduce and form macrocolonies; the remainder of the bacteria comprises the background lawn. The minimum toxic level is defined as the lowest serial dilution at which decreased macrocolony formation, below that of the spontaneous revertant rate, and an observable reduction in the density of the background lawn occurs.) A maximum dose of 1 mg/plate is used when no toxicity is observed. The densities were recorded as normal, slight, and no growth.

Test Format

After we validated our bacterial strains and determined the optimal dosage of the test substance, we began the Ames Assay. In the actual experiment, 0.1 ml of the particular strain of Salmonella (10^8 cells) and the specific dilutions of the test substance were added to 2 ml of molten top agar, which contained trace amounts of histidine and biotin. Since survival is better from cultures which have just passed the log phase, the Salmonella strains are used 16 hours

(maximum) after initial inoculation into nutrient broth. The dose of the test substance spanned a 1000-fold, decreasing from the minimum toxic level by a dilution factor of 5. All the substances were tested with and without S-9 microsome fraction. The optimal titer of the S-9 was determined and 0.5 ml was added to the molten top agar. After all the ingredients were added, the top agar was mixed, then overlaid on minimum glucose agar plates. These plates contained 2% glucose and Vogel Bonner "E" Concentrate (4). The water used in this medium and all reagents came from a polymetric system. Plates were incubated, upside down in the dark at 37° C for 48 hours. Plates were prepared in triplicate and the average revertant counts were recorded. The corresponding number of revertants obtained was compared to the number of spontaneous revertants; the conclusions were recorded statistically. A correlated dose response is considered necessary to declare a substance as a mutagen. Commoner (5), in his report, "Reliability of Bacterial Mutagenesis Techniques to Distinguish Carcinogenic and Non-Carcinogenic Chemical," and McCann et al (1) in their paper, "Detection of Carcinogens as Mutagen in the Salmonella/Mammalian Microsome Mutagenicity Test: Assay of over 300 Chemicals," have concurred on the test's ability to detect mutagenic potential.

Statistical Analysis

Quantitative evaluation was ascertained by the method of Ames (2). They (2) assumed that a compound which causes twice the spontaneous reversion rate and a correlated dose response is mutagenic.

Chemical Analysis

Our information on the chemical analysis of CHR4 can be found in Appendix A and that for CHR6 can be found in Appendix B. The stability of CHR4 and CHR6 under these test conditions has not been determined but assumed to be stable at room temperature.

RESULTS AND DISCUSSION

Throughout this report, the test substances will be referred to by their code numbers.

<u>Substance</u>	<u>Code No.</u>
Methyl N, N' Dihexylethylene Diamine Monocarbamate	CHR4
1,2,3,4-Tetrahydro-6-Methyl-1-(3-Methyl- 1-Oxo-2-Butenyl) Quinoline	CHR6

On 10 August 1982 and 13 August 1982 the toxicity level determinations were performed on CHR6 and CHR4 respectively. For these experiments, all sterility, strain verification, positive and negative controls were normal (Tables 1 and 2). Toxicity was observed after exposure to 100%, 10% and 1% solutions of CHR4 and 100% and 10% solutions of CHR6 (Tables 3 and 4). A 0.1% solution of CHR4 and 1% solution of CHR6 were used as the highest doses.

On 20 August 1982, the Ames Assay was performed on the test substances. In this assay normal results were observed for all sterility and strain verification controls (Table 5). Normal results were also observed for all positive and negative controls (Table 6). Following exposure of the bacteria to the test substances, no incidences of mutagenicity were observed (Tables 7 and 8).

CONCLUSION

The Ames Assay is able to detect frameshift and basepair mutagenic potential. Our results show no evidence of such potential. Therefore on the basis of the Ames Assay, CHR4 and CHR6, both in the presence and absence of metabolic activation, are not mutagenic at the levels tested.

RECOMMENDATION

CHR4 and CHR6 should be tested using other toxicological assays, if efficacy tests prove this compound to be a promising insect repellent.

REFERENCES

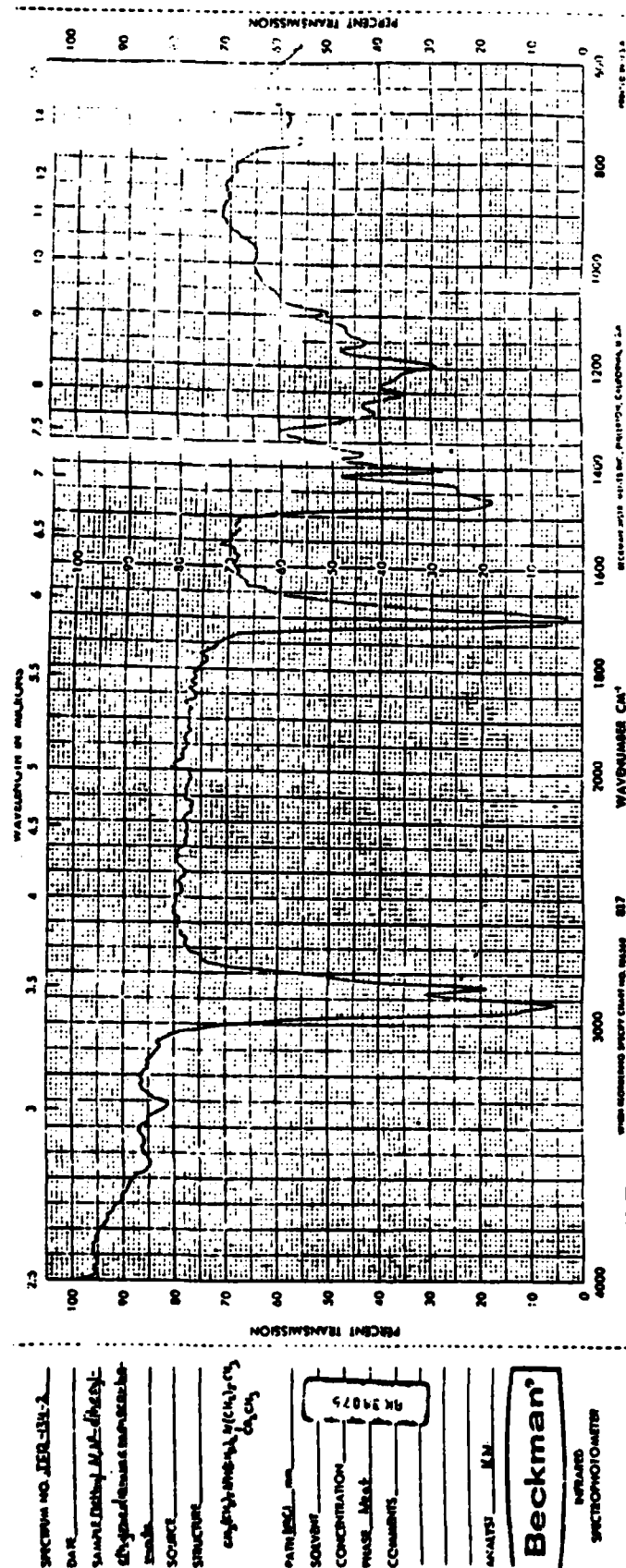
1. McCann JE, Choi E, Yamasaki E, Ames BN. Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc Nat Acad Sci, USA 1975;72:5135-5139.
2. Ames BN, McCann J, Yamasaki E. Methods for detection carcinogens and mutagens with Salmonella/mammalian microsome mutagenicity test. Mutation Res 1975;31:347-364.
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4. Vogel HJ, Bonner DM. Acetylornithinase of E. coli: Partial purification and some properties. J Biol Chem 1956;218:97-106.
5. Commoner B. Reliability of the bacterial mutagenesis techniques to distinguish carcinogenic and non-carcinogenic chemicals. EPA 600/1 76-022, 1976.

Sauers--7

REPORT ON CHEMICAL ANALYSIS FOR CHR4

APPENDIX A

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LIGHT	<input type="checkbox"/>	39	<input type="checkbox"/>		<input type="checkbox"/>																																																																																																						
WATER	54	<input type="checkbox"/>	55	<input type="checkbox"/>	56	<input type="checkbox"/>	CHLOROFORM	76	<input type="checkbox"/>	77	<input type="checkbox"/>																																																																																																
ACID	48	<input type="checkbox"/>	49	<input type="checkbox"/>	50	<input type="checkbox"/>	ETHER	71	<input type="checkbox"/>	72	<input type="checkbox"/>																																																																																																
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ETHANOL	46	<input type="checkbox"/>	47	<input type="checkbox"/>	48	<input type="checkbox"/>		77	<input type="checkbox"/>	78	<input type="checkbox"/>																																																																																																
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EQUATIONS INDICATING SYNTHETIC ROUTE																																																																																																											
$a. \text{C}_6(\text{C}_4)_4\text{COCl} + [\text{N}_2\text{NCH}_2-]_2 \longrightarrow [\text{C}_6(\text{C}_4)_4\text{CONNCH}_2-]_2$ $b. 1 + \text{NaAlH}_2(\text{OCH}_2\text{CH}_2\text{OCH}_3)_3 \longrightarrow [\text{C}_6(\text{C}_4)_5\text{NCH}_2-]_2$ $c. 2 + \text{ClCO}_2\text{CH}_3 \longrightarrow \text{C}_6(\text{C}_4)_5\text{NH}(\text{C}_2)_2\text{N}(\text{C}_4)_5\text{CH}_3$ <div style="text-align: center; margin-top: 10px;"> CO_2CH_3 </div>																																																																																																											
REMARKS *1. CH structure 11.11, 11.12, 11.13, 11.14, 11.15, 11.16, 11.17, 11.18, 11.19, 11.20, 11.21, 11.22, 11.23, 11.24, 11.25, 11.26, 11.27, 11.28, 11.29, 11.30, 11.31, 11.32, 11.33, 11.34, 11.35, 11.36, 11.37, 11.38, 11.39, 11.40, 11.41, 11.42, 11.43, 11.44, 11.45, 11.46, 11.47, 11.48, 11.49, 11.50, 11.51, 11.52, 11.53, 11.54, 11.55, 11.56, 11.57, 11.58, 11.59, 11.60, 11.61, 11.62, 11.63, 11.64, 11.65, 11.66, 11.67, 11.68, 11.69, 11.70, 11.71, 11.72, 11.73, 11.74, 11.75, 11.76, 11.77, 11.78, 11.79, 11.80, 11.81, 11.82, 11.83, 11.84, 11.85, 11.86, 11.87, 11.88, 11.89, 11.90, 11.91, 11.92, 11.93, 11.94, 11.95, 11.96, 11.97, 11.98, 11.99, 12.00, 12.01, 12.02, 12.03, 12.04, 12.05, 12.06, 12.07, 12.08, 12.09, 12.10, 12.11, 12.12, 12.13, 12.14, 12.15, 12.16, 12.17, 12.18, 12.19, 12.20, 12.21, 12.22, 12.23, 12.24, 12.25, 12.26, 12.27, 12.28, 12.29, 12.30, 12.31, 12.32, 12.33, 12.34, 12.35, 12.36, 12.37, 12.38, 12.39, 12.40, 12.41, 12.42, 12.43, 12.44, 12.45, 12.46, 12.47, 12.48, 12.49, 12.50, 12.51, 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REPORT ON CHEMICAL ANALYSIS FOR CHR6

APPENDIX B


DATA SHEET FOR COMPOUNDS																			
DR NO. VR2502021A			SUPPLIER Starks Associates, Inc. 1200 Niagara St. Buffalo, New York 14213				SUBMITTER KEY NO. 0205												
DATE SHIPPED		DAY	MO	YR	DATE RECEIVED		DAY	MO	YR	DATE ACKNOWLEDGED		DAY	MO	YR					
		24	5	82			27	5	82										
NAME OF COMPOUND 1,2,3,4-Tetrahydro-6-methyl-1-(3-methyl-1-oxo-2-butenyl)quinoline												1282							
STRUCTURE <div style="display: flex; align-items: center; justify-content: space-around;"> <div style="text-align: center;"> </div> <div style="text-align: center;"> CH_3 $\text{COCH}=\text{C}(\text{CH}_3)_2$ </div> </div> <p style="text-align: right; margin-top: 10px;"> <i>Ship 1200g to LAIR with all data sheets & spectra alth: Maj. Eisenberg is ship on printing!</i> </p>																			
SOL. FORMULA C₁₅H₁₉NO														MOL WT 229.171		ANALYSES			
APPEARANCE viscous yellow oil						ELEMENT		CALCULATED		FOUND		VR FOUND							
QUANTITY 1260 g						C		78.57		78.43									
NOTES ON REP. JF16-29-1						H		8.35		8.30									
TEST SYSTEM JF16-29-1						N		6.11		6.24									
S.P. 151.5						M.P.				REFRACT. INDEX									
attached spectrum See attached spectrum																			
CHROMATOGRAPHY: Homogeneous (JTB precoated TLC plates, glass support, 5 cm x 10 cm, 0.25 mm silica gel F-254; detection - ultraviolet light.)																			
LITERATURE 1. The compound is unknown to the chemical literature.																			
STABILITY (Check Where Applicable)																			
STABLE UNSTABLE				STABLE UNSTABLE				STABLE UNSTABLE				STABLE UNSTABLE							
ACID	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	BASE	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	HEAT	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	LIGHT	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>				
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SPECTRUM NO. 111-25-1

DATE 1-23-54

SAMPLE 1,2,3,4-Tetramethyl-1,2,3,4-tetrahydronaphthalene

SOURCE 2-Butanol

STRUCTURE 

ANALYST M.H.W.

Beckman INFRARED SPECTROPHOTOMETER

PERCENT TRANSMISSION

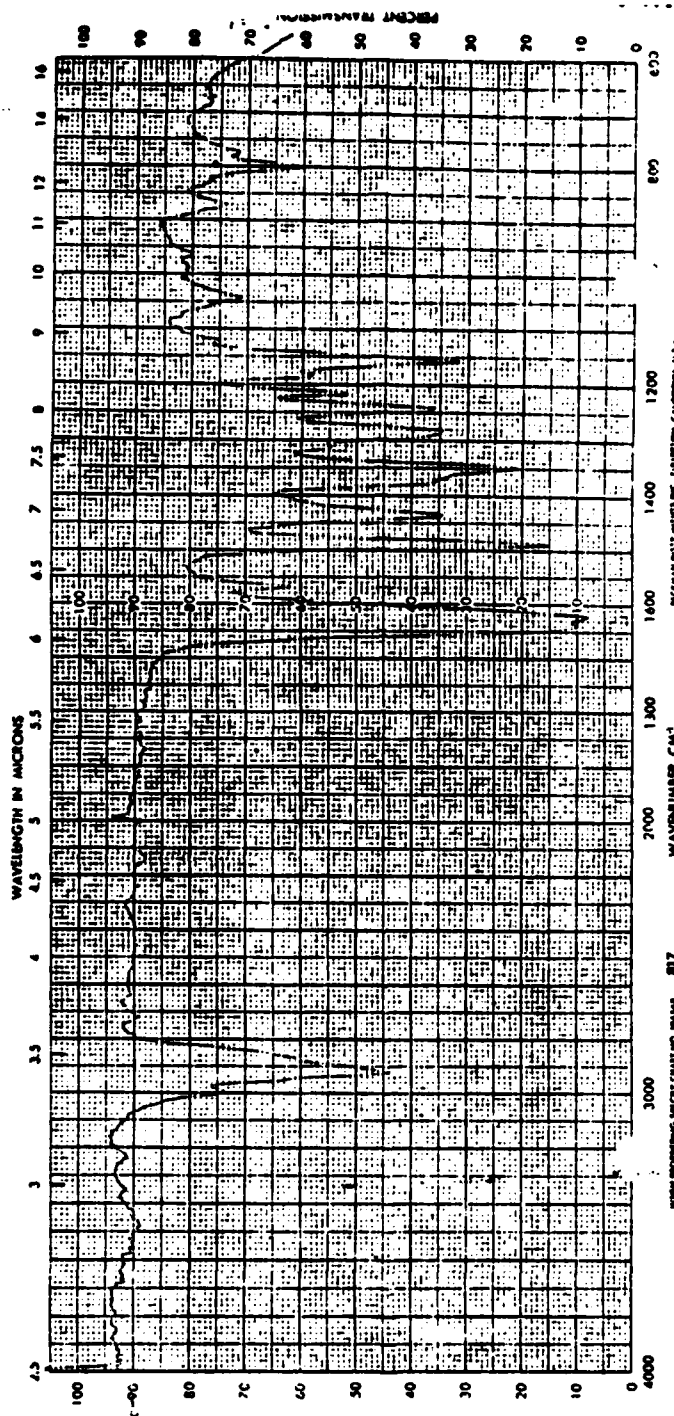
WAVELENGTH IN MICRONS

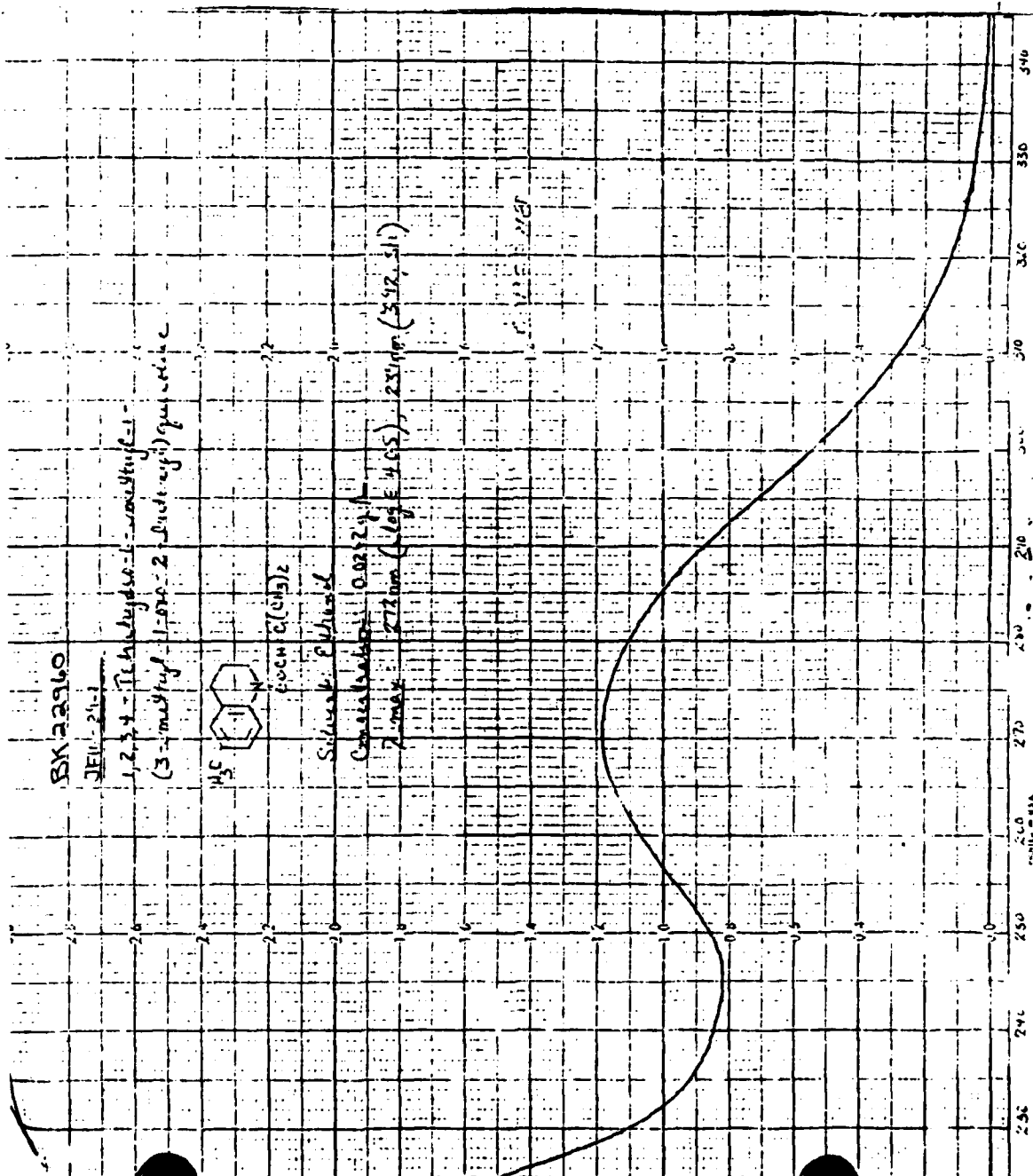
WAVENUMBER CM⁻¹

PERCENT TRANSMISSION

WAVELENGTH IN MICRONS

WAVENUMBER CM⁻¹





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APPENDIX C

TABLE 1

STRAIN VERIFICATION FOR TOXICITY LEVEL DETERMINATION

Strains	Histidine Requirement	Ampicillin Resistance	UV	Sensitivity to Crystal Violet	Sterility Control	Response (1)
100	NG	G	NG	14.5 mm	NG	+
1537	NG	14.5 mm	NG	13.2 mm	NG	+
WT	G	NA	G	G	NA	+

STERILITY CONTROLHis-Bio Mix Initial: NG End: NG MGA Plate: NGTop Agar Initial: NG End: NGDiluent: NG Nutrient Broth: NGTest Compound (a) CMP-NG (b) CHR6-NG (c) NA (d) NA (e) NA

G = Growth NG = No Growth NT = Not Tested NA = Not Applicable WT = Wild Type

Spontaneous Revertants: TA 100, No S-9 109, 95, 81, 97, 121, 109 Average: 102

(1) + = expected response - = unexpected response

Study Number: 82024 Date: 10 Aug 82 By: Sauers

TABLE 2
STRAIN VERIFICATION FOR TOXICITY LEVEL DETERMINATION

Strains	Histidine Requirement	Ampicillin Resistance	UV	Sensitivity to Crystal Violet	Sterility Control	Response (1)
100	NG	G	NG	13 mm	NG	+
1537	NG	14 mm	NG	12.5 mm	NG	+
WT	G	NA	G	G	NA	+

STERILITY CONTROL

His-Bio Mix Initial: NG End: NG MGA Plate: NG
 Top Agar Initial: NG End: NG
 Diluent: NG Nutrient Broth: NG
 Test Compound (a) CHR4-NG (b) NA (c) NA (d) NA (e) NA

G = Growth NG = No Growth NT = Not Tested NA = Not Applicable WT = Wild Type

Spontaneous Revertants: TA 100, No S-9 90, 88, 94 Average: 91

(1) + = expected response - = unexpected response

Study Number: 82024 Date: 13 Aug 82 By: Sauers

TABLE 3

TOXICITY LEVEL DETERMINATION

Substance assayed: CHR4 Substance dissolved in: DMSO
 Study Number: 82024 Date: 13 Aug 82 Performed by: Sauers, Kellner, Dacey, Mullen

TA 100 REVERTANT PLATE COUNT

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	Background Lawn (1)
100% solution	0	0	0	0	NG
10% solution	0	0	0	0	NG
1% solution	0	0	0	0	NG
0.1% solution	62	71	67	67	NL
0.01% solution	80	91	74	82	NL
0.001% solution	76	76	60	71	NL
0.0001% solution	86	60	78	75	NL
0.00001% solution	80	70	62	71	NL

(1) NG = No Growth ST = Slight Growth NL = Normal Lawn

TABLE 4

TOXICITY LEVEL DETERMINATION

Substance assayed: CHR6 Substance dissolved in: DMSO
 Study Number: 82024 Date: 10 Aug 82 Performed by: Sauers, Kellner, Mullen

TA 100 REVERTANT PLATE COUNT

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	Background Lawn (1)
100% solution	0	0	0	0	NG
10% solution	0	0	0	0	NG
1% solution	40	56	71	56	ST
0.1% solution	112	80	94	95	NL
0.01% solution	90	94	94	93	NL
0.001% solution	93	84	88	88	NL
0.0001% solution	86	108	83	92	NL
0.00001% solution	109	116	100	108	NL

(1) NG = No Growth ST = Slight Growth NL = Normal Lawn

TABLE 5

STRAIN VERIFICATION CONTROL

Strains	Histidine Requirement	Ampicillin Resistance	UV	Sensitivity to Crystal Violet	Sterility Control	Response (1)
98	NG	G	NG	12 mm	NG	+
100	NG	G	NG	12 mm	NG	+
1535	NG	NA	NG	14 mm	NG	+
1537	NG	14 mm	NG	13 mm	NG	+
1538	NG	NA	NG	12 mm	NG	+
WT	G	NA	G	G	NA	+

STERILITY CONTROL

His-Bio Mix Initial: NG End: NG Diluent: NG
 Top Agar Initial: NG End: NG MGA Plate: NG
 S-9 Mix Initial: NG End: NG Nutrient Broth: NG
 Test Compound (a) CHR4-NG (b) CHR6-NG (c) CMP-NG (d) NA (e) NA (f) NA

G = Growth NG = No Growth NT = Not Tested NA = Not Applicable WT = Wild Type

Study Number: 82024 By: Sauers (1) + = expected response

Date: 20 Aug 82 - = unexpected response

TABLE 6
NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	98	100	Strain No. 1535	1537	1538
AF	2 ug/plate	yes	(648,518,591) 586	(337,377,285) 333	(579,677,449) 568		
BP	2 ug/plate	yes	(94, 73, 90) 86	(350,361,357) 356	(37, 54, 27) 39	(76, 52, 87) 72	
AA	2 ug/plate	yes	(612,803,656) 690	(999,831,934) * 921	(150,161,171) 161	(909,919,721) 850	
MNNG	2 ug/plate	no		(871,999,999) * 956			
	20 ug/plate	no			(999,999,999) * 999		

Spontaneous Reversion Rate

before	yes	(18, 18, 25) (16, 19, 18)	(89, 91, 74) (93, 92, 89)	(9, 8, 8) (17, 13, 9)	(3, 4, 5) (6, 5, 3)	(15, 19, 11) (9, 12, 22)
after		19	88	11	4	15
before	no	(11, 11, 23) (16, 17, 25)	(77, 60, 69) (63, 87, 75)	(11, 12, 7) (15, 10, 16)	(3, 4, 7) (4, 3, 6)	(14, 7, 10) (13, 14, 10)
after		17	72	12	5	11

*: a value of 999 indicates a colony count of greater than 1000

Study Number: 82024

Date: 20 Aug 82 By: Sauers, Kellner, Lewis, Dacey

TABLE 7

NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	NUMBER OF REVERTANTS/PLATE			
			98	100	Strain Number 1535 1537 1538	
CHR4	0.1% Solution	No	(14, 9, 10) 11	(72, 66, 83) 74	(9, 11, 10) 10	(2, 5, 10) 6 (19, 11, 13) 14
		Yes	(28, 12, 21) 20	(73, 81, 89) 81	(10, 6, 7) 8	(6, 3, 3) 4 (16, 13, 11) 13
CHR4	2 x 10 ⁻² % Solution	No	(18, 10, 16) 15	(72, 72, 67) 70	(10, 14, 8) 11	(2, 3, 5) 3 (5, 16, 12) 11
		Yes	(15, 14, 19) 16	(75, 86, 77) 79	(10, 10, con) 10	(10, 3, 4) 6 (11, 15, 10) 12
CHR4	4 x 10 ⁻³ % Solution	No	(15, 13, 13) 14	(72, 72, 88) 77	(9, 12, 7) 9	(3, 3, 4) 3 (12, 5, 12) 10
		Yes	(19, 28, 18) 22	(73, 60, 83) 72	(6, 10, 10) 9	(7, 5, 4) 5 (9, 19, 11) 13
CHR4	8 x 10 ⁻⁴ % Solution	No	(18, 12, 9) 13	(55, 66, 56) 59	(13, 8, 15) 12	(11, 4, 3) 6 (11, 11, 11) 11
		Yes	(18, 9, 14) 14	(68, 72, 63) 68	(9, 5, 14) 9	(3, 5, 4) 4 (12, 19, 13) 15

con - Plate value disregarded due to contamination

-continued

Study Number: 82024 Date: 20 Aug 82 By: Sauers, Kellner, Lewis, Dacey

TABLE 7 (cont.)

NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number				
			98	100	1535	1537	1538
CHR4	1.6 x 10 ⁻⁴ % Solution	No	(15, 11, 12) 13	(67, 62, 64) 64	(9, 9, 9) 9	(3, 4, 2) 3	(9, 14, 7) 10
		Yes	(11, 14, 20) 15	(54, 56, 56) 55	(12, 8, 7) 9	(6, 3, 4) 4	(11, 12, 14) 12
CHR4	3.2 x 10 ⁻⁵ % Solution	No	(15, 14, 8) 12	(62, 58, 62) 61	(9, 13, 13) 12	(3, 3, 2) 3	(6, 10, 8) 8
		Yes	(25, 21, 17) 21	(91, 81, 79) 84	(13, 8, 15) 12	(3, 5, 4) 4	(11, 20, 10) 14

Study Number: 82024 Date: 20 Aug 82 By: Sauers, Kellner, Lewis, Dacey

TABLE 8
NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	98	100	Strain Number 1535	1537	1538
CHR6	1% Solution	No	Toxic	Toxic	Toxic	Toxic	Toxic
		*Yes	Toxic	Toxic	Toxic	Toxic	Toxic
CHR6	0.2% Solution	No	(11, 15, 13) 13	(80, 80, 73) 78	(14, 6, 19) 13	(5, 4, 4) 4	(11, 12, 12) 12
		Yes	(11, 15, 20) 15	(95, 81, 73) 83	(8, 6, 13) 9	(4, 5, 3) 4	(12, 12, 16) 13
CHR6	$4 \times 10^{-2}\%$ Solution	No	(9, 14, 8) 10	(68, 77, 60) 68	(6, 10, 8) 8	(4, 3, 3) 3	(7, 8, 8) 8
		Yes	(22, 19, 22) 21	(64, 91, 81) 79	(9, 12, 7) 9	(5, 4, 2) 4	(21, 16, 8) 15
CHR6	$8 \times 10^{-3}\%$ Solution	No	(16, 13, 11) 13	(62, 61, 69) 64	(15, 9, 10) 11	(7, 2, 3) 4	(7, 10, 16) 11
		Yes	(34, 19, 16) 23	(76, 68, 70) 71	(9, 10, 10) 10	(6, 7, 5) 6	(20, 16, 9) 15

-continued

*background lawn very sparse, few survivors seen

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TABLE 8 (cont.)

NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number				
			98	100	1535	1537	1538
CHR6	1.6 x 10 ⁻³ % Solution	No	(18, 12, 16) 15	(69, 85, 83) 79	(9, 11, 11) 10	(4, 2, 2) 3	(13, 6, 15) 11
		Yes	(19, 17, 14) 17	(58, 60, 62) 60	(17, 13, 10) 13	(8, 5, 6) 6	(7, 17, 12) 12
CHR6	3.2 x 10 ⁻⁴ % Solution	No	(16, 9, 12) 12	(60, 73, 72) 68	(10, 11, 12) 11	(3, 4, 4) 4	(13, 18, 12) 14
		Yes	(16, 23, 18) 19	(72, 79, 70) 74	(5, 8, 8) 7	(5, 5, 6) 5	(12, 12, 25) 16

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